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# Maine Agricultural Experiment Station

ORONO

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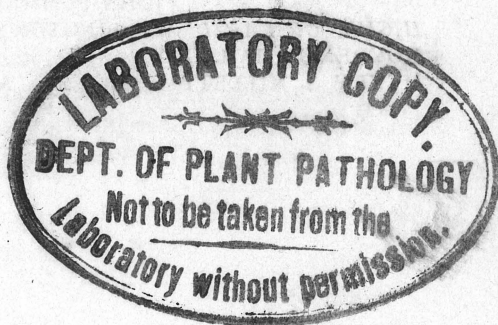
## APPLE DISEASES CAUSED BY

*Coryneum foliicolum* and *Phoma mali*

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This bulletin contains an account of the cultural characters of these two fungi together with the results of inoculation experiments which were made in order to determine the extent of their parasitism upon leaves, wood, and fruit of the apple.

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## BULLETIN No. 170.

### APPLE DISEASES CAUSED BY

*Coryneum foliicolum* Fckl. and *Phoma mali* Schulz et Sacc.

CHARLES E. LEWIS.

Careful examination of the fungi associated with diseases of the apple in Maine shows that they may be divided into classes according to the extent of their parasitism. Some of these fungi have been carefully studied at a number of different places and have been shown to be the cause of disease under the conditions which exist in those localities, others have been repeatedly shown to be saprophytes, others have been either regarded as saprophytes or have not been studied sufficiently to determine to what extent they are parasites, and still others which have been usually regarded as parasites because of their association with diseased conditions are found in some cases to be saprophytes.

Since under certain special conditions, a fungus which is usually saprophytic may take on a parasitic habit and a fungus which is a parasite under one set of conditions may lose the power to cause disease under other conditions, it becomes necessary to study the fungi associated with the diseases of any host plant in a given locality as to their ability to cause disease. In the studies of apple diseases which are now under way at the Maine Experiment Station, fungi have been isolated from diseased leaves, wood, and fruit and these fungi are being studied as to the extent to which they cause disease when the different parts of the plant are inoculated from pure cultures.

Most of the results of this work will be given in later publications but at the present time it seems desirable to give the results of the study of two fungi. In the literature there is very little reference to either of these as a cause of disease, although each belongs to a genus in which there are species which are parasites of great economic importance.

*Coryneum foliicolum* Fckl.

This fungus occurs so commonly on leaf-spot of the apple as to suggest the possibility that, in some cases, it may be the cause of that disease. This species seems to be more or less widely distributed as it is reported as common in West Virginia by Hartley (3) and in New Hampshire by Lewis (4). Hartley suggests that it is probable that the fungus which has been reported from several states as *Hendersonia mali* Thüm, is really *Coryneum foliicolum* Fckl. and, if this be true, the distribution of the fungus would be considerably widened. It would be very easy to confuse the two species unless sections were prepared to show how the spores are borne as the spores are very similar. On a single leaf-spot several of the fruiting pustules of the fungus may be found. Spores are produced from these pustules in large numbers and become piled up in black carbonaceous masses which somewhat resemble pycnidia. When sections of these pustules are examined, however, it is found that the fungus belongs to the Melanconiales as shown by Figures 28 and 29.

Hartley (3) has made some study of *Coryneum foliicolum* in connection with a more thorough study of *Coniothyrium pirina* (Sacc.) Sheldon. He grew the fungus in pure culture and made some inoculations but came to the conclusion that *Coryneum* is less actively parasitic than *Coniothyrium*.

On account of the frequent occurrence of this fungus on leaf-spot in Maine orchards in 1908, the writer felt that further investigation was desirable. This seemed more necessary when it was found that spores similar to those found on the leaf-spots occurred very frequently in cankers on apple branches. Cultures from these spores showed that the fungus in the cankers and the one on the leaf-spots were identical.

## INOCULATION EXPERIMENTS.

Experiments were carried on to determine the extent of parasitism of the fungus on leaves, wood, and fruit of the apple. Material from pure cultures was used in making all inoculations. The abundant production of spores in culture, and the fact that the spores germinate in a few hours in water should make this fungus a favorable one to use in infection experiments.



For inoculation of leaves, seedlings grown in the greenhouse, trees one year from the bud brought into the greenhouse early in the spring and grown in pots, and both old and young leaves on branches of bearing trees in the orchard were used. Sterile water containing an abundance of the *Coryneum* spores was sprayed on the leaves with an atomizer. Seedling trees 3 to 4 months old bearing a few leaves were placed in a moist chamber and kept there for a few days after inoculation. The year old trees were also in some cases placed in a moist chamber made by using a tall bell-jar arranged as shown in Fig. 17 with tubing so connected that water dropped on sheets of blotting paper under the bell-jar at such intervals as to make a moist atmosphere.

As a result of these inoculation experiments, it was found that *C. foliicolum* did not grow on uninjured leaves. When spots in the leaves were killed with a heated needle it was found that the fungus developed readily on the dead spots. One week after inoculation of such leaves there were numerous masses of spores on the dead spots which microscopic examination showed to be spores of *Coryneum*. Observations of these leaves for several weeks showed that these spots did not increase in size by the invasion of the healthy tissue by the fungus mycelium. Neither did infections occur on other healthy leaves from spores produced on the dead spots.

The results of these inoculation experiments are made more valuable by the fact that, at the same time that they were being carried on, the writer studied a number of other fungi commonly associated with leaf-spot. *Phyllosticta limitata* Pk., *Coniothyrium pirina* (Sacc.) Sheldon, *Sphaeropsis malorum* Pk., and a species of *Phoma* probably *Phoma mali* Schulz. et. Sacc., were tested as to their ability to cause leaf-spot, and of these it was found that only *Sphaeropsis malorum* Pk. caused the disease on uninjured leaves although the other fungi developed readily on dead spots in the leaves.

The first inoculations made to determine whether *C. foliicolum* could develop on living apple wood and thus cause cankers were made May 4, 1909, on a young tree growing in a pot in the greenhouse. Incisions were made in the bark on the branches in 7 places and material of the fungus consisting of both mycelium and spores from a young culture

were placed in the incision. These places were then wrapped in moist absorbent cotton to prevent drying out.

One week after the time of inoculation, it could be noted that there was some evidence of growth and at the end of 10 days there was a region of dead sunken bark around each of the inoculated places. The fungus continued to invade the healthy tissue to such an extent that May 20, 16 days after the inoculation, the branches were almost girdled in some places and completely girdled in others. The regions injured by the fungus were 2 to 7 cm. in length. The leaves on the girdled branches were wilted while those on uninoculated branches were green as shown in Fig. 18. Fig. 19 shows two of the cankers enlarged. On the dead bark little black pustules were observed which on examination proved to be the fruiting pustules of *Coryneum foliicolum*. Sections through these pustules were prepared from which Figs. 28 and 29 were taken.

A piece of one canker was cut off and placed in 1-1000 corrosive sublimate for two minutes after which it was thoroughly washed with sterile distilled water. Eight pieces were cut from this canker at various places with a sterile scalpel and placed in plates of prune agar. After one week, the plates were examined and it was found that pure cultures of *Coryneum* had grown from 5 of the pieces while the others showed *Coryneum* which was accompanied in one case by *Alternaria*, in another by *Coniothyrium pirina* (Sacc.) Sheldon and in the third by what is probably a species of *Torula*.

Two of the one-year-old trees in the greenhouse were inoculated at 4 places each May 13, and two others in 4 places each May 18, the same method being followed that was used in the first set of inoculations. One check incision was made in each of the 4 trees. All of the places which were inoculated developed well marked cankers. The small branches were girdled and killed in from 2 to 4 weeks but inoculations on the main stem did not girdle and kill the tree in either case although considerable areas of bark were killed. The check incisions to which no fungus was added soon began to heal over by the formation of callus.

For comparison, inoculations were made May 13 with *Sphaeropsis malorum* on 5 trees which were kept under the same conditions as those which were used for *Coryneum foliicolum*.

*Sphaeropsis* did not spread more rapidly than *Coryneum* and did not do greater damage to the young trees. In each case, some of the inoculations resulted in girdling the branch and killing the part above it, and, in some other cases with each, the spread of the fungus was checked by the development of callus. Some inoculations were also made with *Coniothyrium pirina* (Sacc.) Sheldon. This fungus did not invade the healthy tissue but grew to some extent in bark which was injured by the inoculations. In this my results agree with those of Hartley (3) who carried on extensive inoculation experiments with this fungus. *Phyllosticta limitata* Pk., *Cylindrosporium pomi* Brooks, and *Epicoccum granulosum* Penz. gave negative results, the wounds healing over almost as readily as the checks.

*Glomorella fructigena* (Clinton) Sacc. was also used in making inoculations on 7 of the one-year-old trees in the greenhouse. Edgerton (2) has pointed out that there are two forms of this fungus, northern and southern, and that they differ in cultural characters. Inoculations made with material from cultures which agree with the southern form in the development of perithecia, both in culture and on the inoculated trees, show that this form is much more actively parasitic on the young trees than *Coryneum foliicolum* or *Sphaeropsis malorum*. The cultures of this form were obtained from an apple from a grocery in Orono. The northern form which was isolated from both apples and cankers collected out of doors in Orono, grows slowly in culture, has not produced perithecia either in culture or in the cankers and does not spread so rapidly nor do so much damage to the young trees upon inoculation. Nothing has been done in comparing the two forms with *Coryneum* by inoculation of older trees in the orchard but it is expected that this can be done next year.

In order to determine the extent to which *Coryneum foliicolum* is capable of causing disease of branches of apple trees in the orchard, inoculations were made May 21, 1909, in branches one to 3 cm. in diameter on bearing trees. The inoculations were made in the same manner that has been described for the young trees, the inoculated places and check incisions being wrapped in moist absorbent cotton. On the day following the inoculations the cotton was wet and two days later rains and cloudy weather began which lasted two days. Examina-

tion two weeks later, showed that of the 20 places inoculated, in all except 2, which seemed doubtful, the fungus was growing and invading the uninjured tissue giving the bark a brown color and a somewhat sunken appearance. Observations from time to time through the summer showed that cankers were developing. The bark was killed in regions 3 to 5 cm. in length which in some cases had almost girdled the branch by September 1. The fungus began to fruit on the dead bark in 3 to 4 weeks after the inoculations. The cankers shown in Fig. 20 were removed September 1. The bark on the affected region has a sunken appearance which comes about through the death of the cambium cells so that the wood and bark cease to grow. There was no dying of bark nor cankered appearance from check incisions nor from inoculations made with *Coniothyrium pirina*.

June 24, 1909, inoculations were made on large branches of Ben Davis and Baldwin trees. *Coryneum*, *Sphaeropsis* and *Phoma* were used. 16 inoculations were made with *Coryneum*, 6 with *Sphaeropsis*, and 11 with *Phoma*. 6 check incisions were made. When these trees were examined September 30, it was found that the checks were almost healed over by callus so that the appearance was healthy. *Sphaeropsis* had spread into the uninjured tissue and had formed well marked cankers in each case. *Coryneum* had spread to some extent in 14 of the 16 places but had almost healed over in the other 2. The places inoculated with *Phoma* showed about the same as has been described for *Coryneum*, one place was almost healed over and the others had spread to some extent. One point of importance which remains to be determined is the extent to which these cankers caused by *Coryneum* will spread from year to year. It is intended to keep some of this year's cankers under observation so that this question may be answered.

The results of the study of *Coryneum foliicolum* by means of inoculations of living apple trees show that this fungus is a parasite which is capable of doing great damage to young trees and the small branches of older trees. It has also proved able to keep wounds on larger branches from healing but it will be necessary to keep such branches under observation for a longer time to determine the extent of the injury.



In connection with the statement that *Coryneum foliicolum* is a parasite it is of interest to compare the parasitism of two other species of *Coryneum* which are of great economic importance. *C. beyerinckii* Oudem. has been reported by a great many investigators in widely separated places as causing diseases of stone fruits. Recently, Smith (5) has made a careful study of "Peach Blight" in California and shows that the disease is caused by this fungus which attacks both the leaves and twigs. The mycelium of the fungus is able to penetrate the bark of new shoots and kills small areas, causing spots. Spores lying about the bud scales produce mycelium which penetrates and kills outright both the bud and the surrounding bark, the spot extending from one-fourth to one inch in length.

Butler (1) gives an account of a disease of the mulberry in India caused by *Coryneum mori* Nom. This parasite attacks young trees in the nursery and the smaller branches of full grown trees. It enters through injured places in the bark but is not able to penetrate the uninjured bark. The treatment recommended for this disease is to avoid the making of unnecessary wounds, to prune in such a way as to make wounds which will heal over readily, and to burn dead and diseased wood after it has been removed.

*Coryneum foliicolum* agrees quite closely with *Coryneum mori* in the manner of its attack on the host. In the inoculations for leaf-spot, where large numbers of spores were sprayed upon the young branches, no case was observed in which the fungus penetrated the bark and caused disease, therefore it seems probable that the fungus is able to enter only through wounds. The control of such a disease caused by a wound parasite should not be a difficult matter. The same methods carefully applied would also go far toward controlling diseases caused by certain other fungi. All dead and diseased wood should be removed and burned, as this would destroy to a large extent the material for infection. Care should always be taken to avoid unnecessary wounds or wounds which will not readily heal over.

In connection with a study of apple decays, the writer has isolated fungi from a large number of decaying apples both by the poured plate method and by taking out material from decaying apples from regions which were either some distance

from the point of infection or were on the border line between the decayed and the undecayed tissue with a scalpel which had been sterilized by heat and transferring to plates of agar. In no case has *Coryneum foliicolum* been isolated from a naturally infected apple.

In order to test the ability of the fungus to cause decay, two ripe apples were inoculated October 31, 1908. Only a very small amount of decay developed but that this decay was caused by *Coryneum* was proved by reisolating the fungus in pure culture from the decaying tissue.

Six green apples were inoculated August 24, 1909. The fungus grew to a slight extent at points of inoculation but did not spread into surrounding tissue to cause decay.

Three ripe apples were inoculated October 5, 1909. A slow decay took place. At the end of two weeks the decayed region was about 1.5 cm. in diameter, and increased very slowly after that time.

Three ripe pears were inoculated September 16. There was a little growth at the points of inoculation but the fungus did not spread to cause much decay.

These inoculations show that *Coryneum* can cause a slow decay of ripe fruit but when the decay caused by this fungus is compared with that caused by such fruit decaying fungi as *Sphaeropsis* and *Penicillium* it is seen that *Coryneum* is not of much importance as a fruit decay.

#### CULTURAL STUDIES.

When the spores of *Coryneum foliicolum* from either leaf-spot or canker are placed under favorable conditions for growth they germinate readily. In hanging drops of sterile, distilled water nearly all of the spores had germinated at the end of 16 hours at 65° to 70° F. The cells become swollen and rounded and germ tubes are produced from one or more cells as shown by Fig. 35. About the same characters are shown by spores germinated in hanging drops of prune decoction. When spores are sown in dilution plates of prune or bean agar, the germ tubes have begun to branch by the end of 24 hours and in 4 or 5 days the mycelium has begun to produce spores after the manner of the Hyphomycetes as shown in Figures 27, 33 and 34. If this fungus were classified according to its characters

when grown on prune agar, it would belong to the genus *Clasterosporium* of the Dematiaceæ.

The fungus has been grown on a number of the common culture media. It grows well and produces spores abundantly in plates of prune agar, bean agar and potato agar. Considerable ærial mycelium is produced in each case which differs in color on the different media being almost white on bean and potato agars and dark brownish gray on prune agar. The central part of the colony is wet and slimy in each case with no ærial mycelium. The spores are produced in such numbers as to form dense black masses. In the bean agar plates the black spore masses were formed in concentric rings. The fungus seems to fruit just as well when the colonies are crowded in the petri dish as when only one colony is present. Plates of prune agar were sown with spores so that from 30 to 100 colonies developed in a plate. The colonies did not merge together but were separated by quite clear-cut lines where they approached each other. The ærial mycelium was well developed and these colonies formed spores.

The mycelium of the fungus consists of large threads from which finer branches are given off and is shown well by Fig. 37 which is a photomicrograph of mycelium from a prune agar culture. The hyphæ vary in width from 1.5 to 8 microns, and the length of the cells varies greatly, being from 11 to 80 microns for the most part although, in some cases, the actively growing terminal end of a hypha is seen which shows no cross wall in more than 200 microns. Not much difference can be noted in structural characters of the mycelium on different culture media as measurements of hyphæ from a number of media gave the same results.

There is, however, considerable variation in the spores in culture as has been pointed out by Hartley for prune agar cultures. Smith (5) has shown that the same kind of variations takes place in the spores of *C. beyerinkii* and Butler (1) has described and figured the same thing for *C. mori*. The spores of *C. foliicolum* taken from either apple leaf-spot or canker do not vary greatly in size or number of cells. They are for the most part four celled and measure  $4-5.5 \times 13-16.5$  microns. On sterilized bean pods, potato cylinders, and apple twigs in tubes, the spores are for the most part typical

four-celled spores which do not differ from the spores developed under natural conditions. On potato agar in petri dishes, the spores are four celled but vary in size. On prune agar, bean agar, and in prune decoction, the spores vary in size and number of cells as shown in Figs. 31 and 32, all gradations can be easily found from typical four celled spores of the size found under natural conditions to those having as many as nine cells and measuring  $9 \times 43$  microns.

In prune decoction, the fungus grows rapidly and when the cultures were 5 days old a thick pellicle was formed over the surface of the liquid and large numbers of spores were being produced. On sterilized apple twigs, the fungus grows well forming a considerable amount of ærial mycelium which is brownish gray in color, some of the large hyphæ being deep brown in color. The mycelium also grows in the liquid at the bottom of the tube. Spores are produced in black masses on the wood. When the spores are mature, they are mostly broken off from the stalks on which they are borne, but in some cases they were found still attached and the stalks measured 18-22 microns in length. On sterilized bean pods, potato, carrot, and turnip cylinders, there is a considerable development of ærial mycelium which is light colored in young cultures but becomes dark in old cultures. Part of the spores examined from turnip and carrot cultures were very abnormal, the cells being large and rounded and in some cases separated resembling the conditions seen in germinating spores.

Smith (5) has made a study of the cultural characters of *Coryneum beyerinckii* Oudem. He found that spores of that species taken from the bark or from leaves did not grow readily in plate cultures and that made it somewhat more difficult to isolate the fungus in pure culture, but after colonies were obtained, the fungus grew very well producing a little mycelium and thick black crusts of spores on prune agar. This differs from *C. foliicolum* in which spores taken from old cankers in the spring before the leaves open germinate in considerable numbers when sown in petri dish dilution cultures in prune agar or in bean agar. When such spores were placed in hanging drops of prune decoction, about one-half had germinated at the end of 18 hours at room temperature.



No other means of reproduction than by conidia has been observed either in culture or in nature. Smith did not find the perfect stage of *C. beyerinckii* Oudem. although Vuillemin (6) has described a species of *Ascospora* (*A. beyerinckii* Vuil.) which he considered to be the perfect form of *C. beyerinckii*.

*Phoma mali* Schulz. et Sacc.

Another fungus which was isolated from leaf-spots from several sources during the summer of 1908 has been studied in some detail. This fungus did not occur with enough prominence on the leaves to suggest that it caused the disease but it was kept in culture for later study. The fungus did not fruit in culture for several months, although it grew very readily. In the fall of 1908, a fungus was isolated from decaying apples which agreed in cultural characters with the one from leaf-spot.

Apples were inoculated October 31, 1908, with material from a culture of the fungus from leaf-spot. Only a few days were required to prove that the fungus was able to produce a distinct apple decay which spread at about the same rate as the decay caused by some of the well known apple decay fungi. In order to prove that the fungus with which the apples were inoculated was the cause of decay, plates were made for reisolation by taking material from the decaying tissue with a sterilized scalpel and transferring it to plates of agar. All the plates produced pure cultures.

Figure 22 shows the extent of the decay in an apple two weeks after inoculation. Figure 23 is from the same apple cut open and shows that it was almost half decayed at that time. The condition of an apple 34 days after inoculation is shown by Fig. 24. The apple is completely decayed, is somewhat shrunk and wrinkled and numerous pycnidia as well as a considerable amount of white mycelium are seen on the surface. Microscopic examination showed that the pycnidia contained large numbers of hyaline, one-celled spores, which were two guttulate and measured  $2.5-3 \times 5.5-8$  microns. Cultures were made from this apple both by making dilution plates using spores from a pycnidium and by transferring decayed tissue from the inside of the apple. In both ways pure cultures were secured and it was shown that the pycnidia belonged to the

fungus with which the apples were inoculated. Material from the apple shown in Fig. 24 was fixed, sectioned, and stained and the photomicrographs shown in Figures 39, 40 and 41 were made from sections of the pycnidia.

When this fungus was grown on prune agar, in petri dishes the mycelium spread rather rapidly and in a few days had spread over the entire surface. Considerable white aerial mycelium was produced. When the cultures were a few days old, little masses of closely interwoven hyphæ began to appear which were arranged in concentric circles. These gradually increased in size and later drops of clear liquid exuded from them. The appearance of the fungus in plate cultures is shown by Fig. 38 which is from a culture 11 days old growing on prune agar from a piece of the decaying apple described above. At first, it was thought that the little masses of hyphæ were early stages in the development of pycnidia but repeated examination failed to confirm this as no bodies resembling pycnidia and no spores were found.

The mycelium consists of hyphæ 5-7 microns in diameter which in plate cultures radiate out from the center giving off finer branches. The closely interwoven network made up of the finer branches includes hyphæ some of which are less than 2 microns in diameter. The length of the cells varies from 10 to 35 microns in the large hyphæ but terminal cells 50-60 microns in length have been seen in the small hyphæ.

The fungus has been grown on only a few common culture media. The growth on bean agar is very similar to what has been described for prune agar. On sterilized bean pods, the growth is good. The whole bean pod becomes covered by the mycelium and a fine, white, cob-webby aerial mycelium develops. After the fungus had been grown for several months on bean pods, the transfers being made each month, it began to produce pycnidia. The pycnidia here had very much the same appearance as those on the apple shown in Fig. 24. In some cases the pycnidia develop singly and in other cases 2-4 pycnidia develop in a sort of stroma. They always extend above the substratum and are usually covered by fine white hyphæ. When the pycnidia are mature, the spores exude through an opening at the apex and adhere together in long chain-like

masses. The spores agree in shape, size and appearance with spores from the pycnidia on decaying apple. On sterilized potato cylinders in tubes after 5 days, the slants were covered by the mycelium and some white ærial mycelium had developed. After 9 days small compact masses could be noted from which drops of clear liquid were exuding. Twenty days from the time of inoculation, mature pycnidia were found from which the spores were exuding in the manner described for bean pod cultures. On sterilized apple wood the fungus grew slowly producing a thin network of mycelium over the wood and later a small amount of white ærial mycelium. The mycelium did not extend much into the liquid but formed a rather thick crust-like pellicle. Where the mycelium came in contact with the liquid, the hyphæ took on a light brown color. Three weeks after the tubes were inoculated a few pycnidia had developed on the wood and compact masses of hyphæ such as have been described as occurring on other media were seen.

In June, 1909, small apple branches which were dying back were collected in an orchard in Orono and also in Monmouth. On examination, a fungus was found which agreed in its characters with the one described above.

In order to determine the extent to which the fungus could cause disease of the wood, 2 of the young apple trees such as were used in the work with *Coryneum* were inoculated with material from pure cultures growing on bean pods, May 17, 1909. Nine places were inoculated and 2 check incisions were made. One week after the time of inoculation, it could be noted that the fungus was growing at all the places which had been inoculated except one which was as clear and bright as the checks. June 2, there were areas of dead brownish bark around the 8 inoculated places. June 9, it was noted that the areas of dead bark had increased to some extent; and that the cankers resembled those caused by *Coryneum*. A more careful examination showed that a few pycnidia had developed on the dead bark near the incisions and the spores in these pycnidia agreed with spores from decayed apple and from bean pod cultures. June 17, some of the branches were almost girdled but in some cases callus was forming which checked the spread of the fungus to some extent. August 2, the cankers were 2-5 cm. in length and a considerable number of pycnidia were found

on the dead bark. Some sections of these pycnidia were prepared and from one of these sections Fig. 42 was taken. The pycnidia develop in the bark and when they are mature the outer layers of the bark are ruptured and the pycnidia appear above the surface.

Two more young trees were inoculated May 18 in the same way and gave practically the same results.

In order to test the parasitism of the fungus on old trees in the orchard, 12 places were inoculated June 4. In some cases, an inoculation was made in a branch which had been inoculated with *Coryneum*. This fungus did not form as well marked cankers on these branches as *Coryneum* but showed that it was able to grow as a parasite. Eleven places were inoculated on branches of Ben Davis and Baldwin trees in the orchard June 24. All but one of these places developed small cankers.

Attempts were made to infect apple leaves with spores from pycnidia from bean pod cultures using the same methods which were used with *Coryneum*. In no case was there any evidence that the fungus attacked the living leaves.

It has already been shown that this fungus causes a decay of ripe apples. It is well known that some of the fungi which cause decay of ripe apples cannot cause decay of green fruit. To determine the effect of this fungus, 8 green apples were inoculated August 10, 1909. The fungus grew at the points of inoculation but the decay did not spread to a great extent in any of the apples except one. In this apple, some other fungus may have assisted in the decay but the pycnidia of the fungus with which it was inoculated appeared on the surface. The other 7 apples showed at the end of one month a small decayed region about .5 cm. in diameter as illustrated by Fig. 25.

In September and October, 1909, inoculations of ripe pears and of apples which were ripe but not over-ripe were made using material of the fungus which had been carried in culture for more than a year. The pears decayed rapidly and the fungus mycelium broke out over the surface of the decayed part. After 10 days, pycnidia in large number were found among this mycelium. The decay of the apples took place at about the same rate that has been described for the apples inoculated in 1908.



From my study of this fungus in nature, in culture, and by inoculations of fruit and wood of apple it shows the characters of the genus, *Phoma*. Several species of *Phoma* are described as occurring on apple branches. *Phoma pomarum* Thüm. occurs on the fruit of the apple in Europe but the spores of that species do not agree with the spores of the fungus under consideration. *Phoma mali* Schulz. et Sacc. is described as having spores 8 microns in length. *Phoma ambigua* (Nits.) Sacc. agrees closely with *Phoma mali* having spores  $8 \times 3$  microns. It has seemed best to the writer to refer this fungus to *Phoma mali* Schulz. et Sacc. depending on the description which has been given of the cultural characters and of the effects on inoculated apples to enable other students of apple diseases to determine whether or not they are working with the same fungus.

#### SUMMARY.

*Coryneum foliicolum* and *Phoma mali* cause disease of the wood of young apple trees and of branches of old trees. These fungi are more actively parasitic than *Coniothyrium pirina*. As wound parasites, they attack young trees in such a way as to do as much damage as *Sphaeropsis malorum* but do not spread so rapidly in the large branches of older trees.

*Coryneum* causes only a slight decay while *Phoma* causes a rather rapid and complete decay of ripe apples and can attack the green fruit to some extent.

Neither of these fungi has been found to cause disease of uninjured leaves, but, in common with a number of other fungi, they occur on dead spots in apple leaves.

The distribution of these fungi can be largely controlled by removing and burning the dead wood on which they occur.

#### LITERATURE CITED.

1. Butler, E. J. The Mulberry Disease Caused by *Coryneum mori* Nom. with Notes on Other Mulberry Diseases. Memoirs of the Department of Agriculture in India. Vol. II, No. 8, pp. 1-11. 1909.
2. Edgerton, Claude Wilbur. The Physiology and Development of some Anthracnoses. Bot. Gaz. 45: p. 405. 1908.
3. Hartley, Carl P. Some Apple Leaf-spot Fungi. Science N. S. 28. 157-159. 1908.

4. Lewis, Isaac M. Apple Leaf-spot. Report of the New Hampshire Agricultural Experiment Station 20: 365-369. 1908.
5. Smith, Ralph E. California Peach Blight. Cal. Expt. Station Bulletin 191: 73-98. 1907.
6. Vuillemin. Titres et travaux scientifiques. 1890. Reference in Tubeuf and Smith, Diseases of Plants, p. 211.

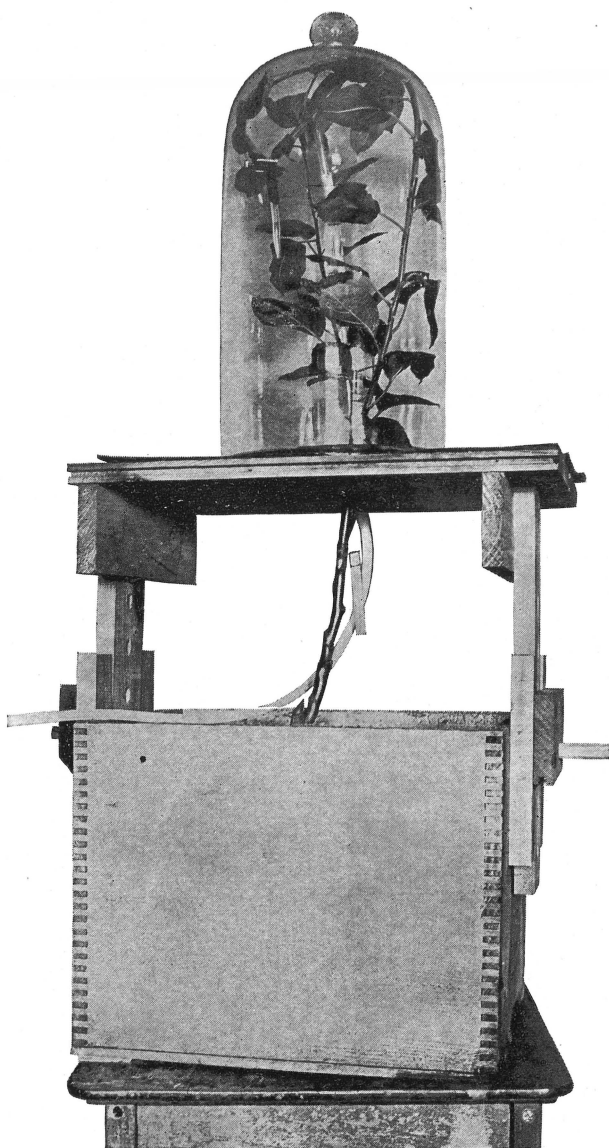


FIG. 17. Moist chamber used in part of leaf-spot inoculations.







FIG. 18. Young apple tree 22 days after inoculation of branches with *Coryneum*. Note the wilted leaves on upper branches.





FIG. 19. Cankers on the branches of the tree shown in Fig. 18.  
Enlarged.







FIG. 20. Cankers produced on branches of tree in orchard by inoculation with *Coryneum*.



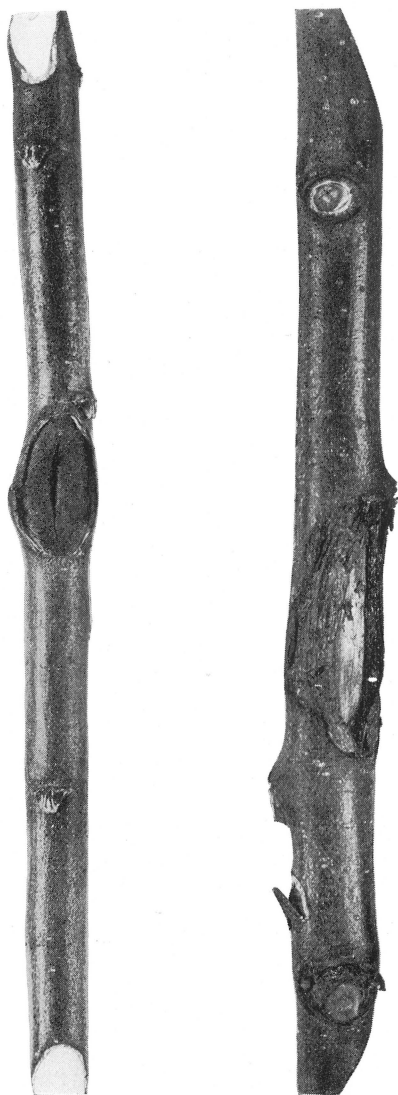


FIG. 21. At left, check incision which  
is healing over. At right, branch  
which was inoculated with  
*Phoma*.







FIG. 22. Apple two weeks after inoculation with *Phoma mali*.

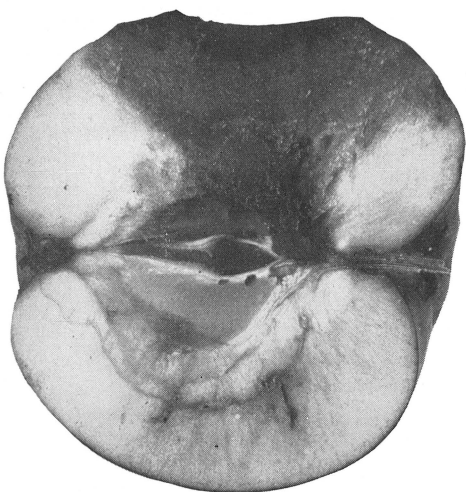


FIG. 23. Same apple cut in two to show extent of the decay.



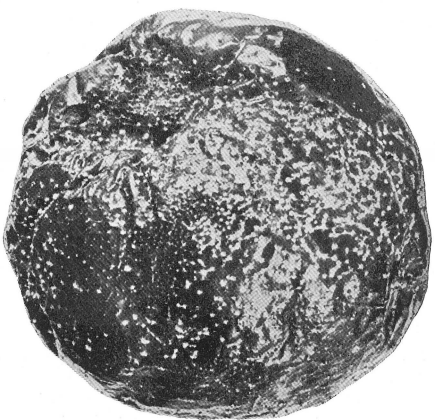


FIG. 24. Apple 34 days after inoculation with *Phoma*. Note pycnidia on surface.

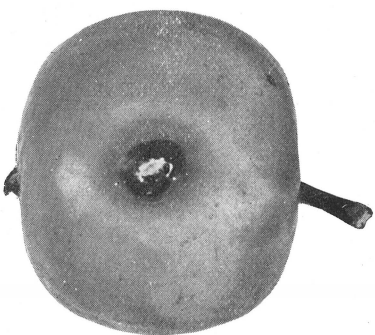


FIG. 25. Green apple 3 weeks after inoculation with *Phoma*.

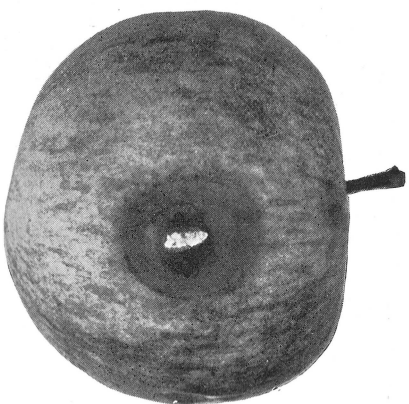


FIG. 26. Ripe apple 2 weeks after inoculation with *Corynesum*.





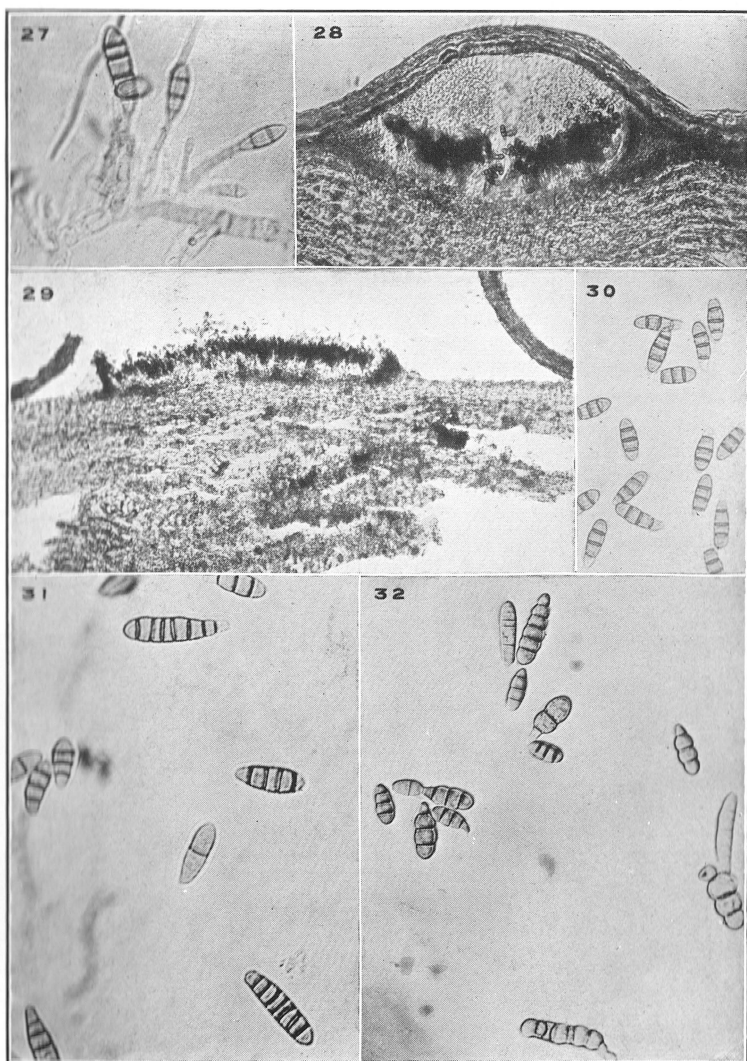


FIG. 27. Spores of *Coryneum* from prune agar culture showing stalks on which they are borne.  $\times 475$ .

FIG. 28. Young fruiting pustule of *Coryneum foliicolum* from canker on tree shown in Fig. 18.  $\times 100$ .

FIG. 29. Old pustule from same source as Fig. 28 in which covering of bark has broken away.  $\times 70$ .

FIG. 30. Spores of *Coryneum* from canker.  $\times 425$ .

FIG. 31. Spores of *Coryneum* from prune agar culture.  $\times 475$ .

FIG. 32. Spores of *Coryneum* from bean agar culture.  $\times 350$ .



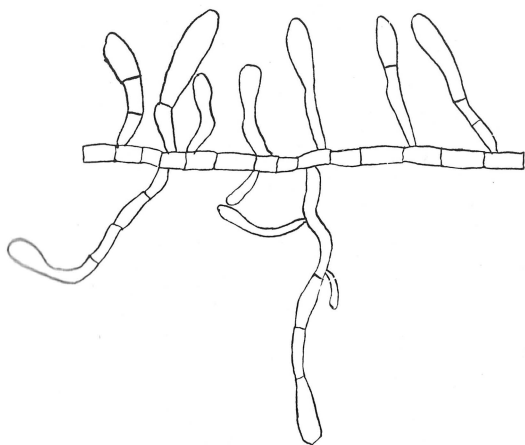


FIG. 33. Developing spores of *Coryneum* in prune agar culture. x 480.

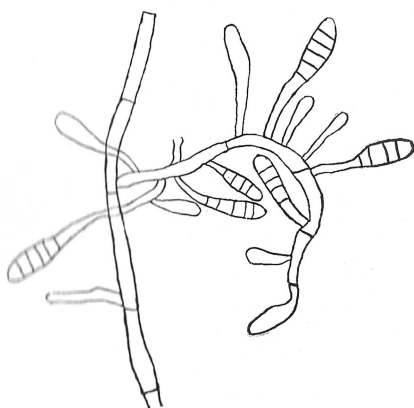


FIG. 34. *Coryneum* from prune agar culture showing how spores are borne. x 480.





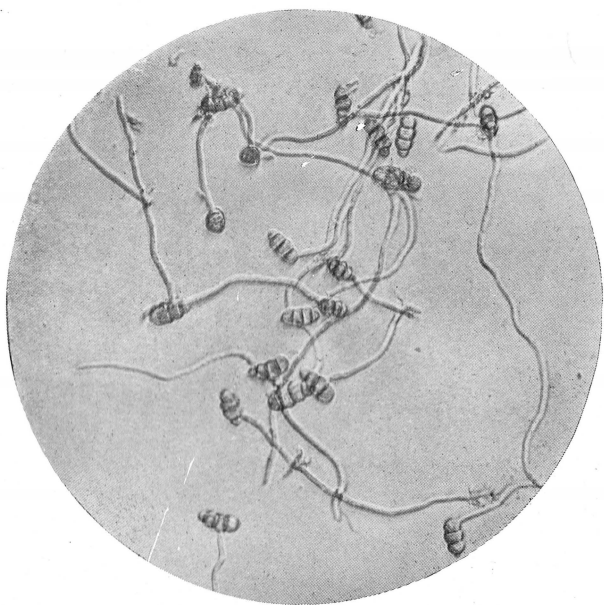


FIG. 35. Germinating spores of *Coryneum* from hanging drop, sterile distilled water. x 300.

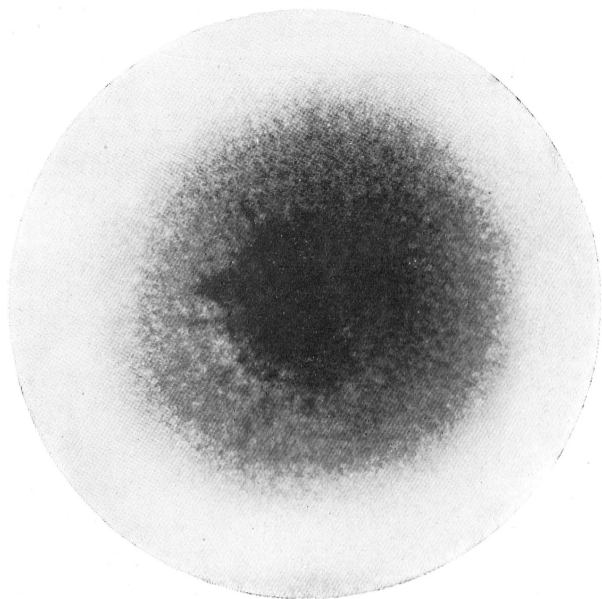


FIG. 36. Colony of *Coryneum* 12 days old on prune agar.



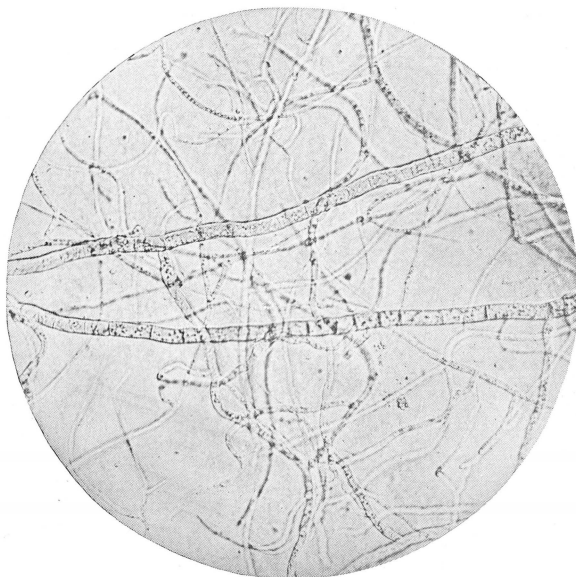


FIG. 37. Mycelium of *Coryneum*. x 300.



FIG. 38. Plate culture of *Phoma mali* 11 days old growing on prune agar.



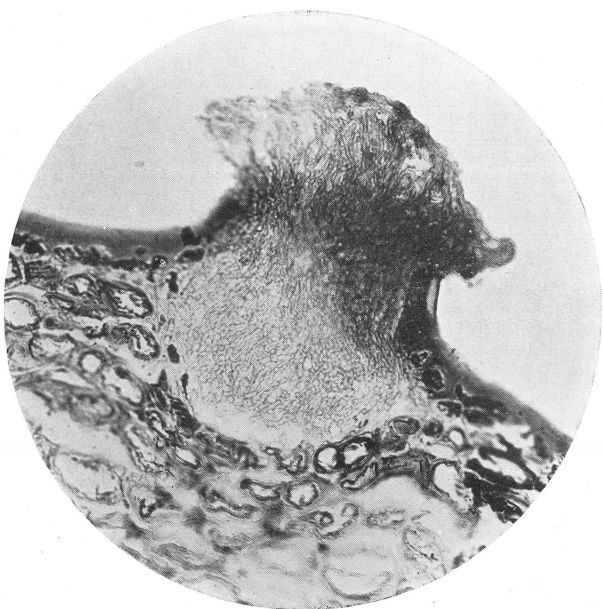


Fig. 39 Young developing pycnidium of *Phoma* from the apple shown in Fig. 24. x 180.

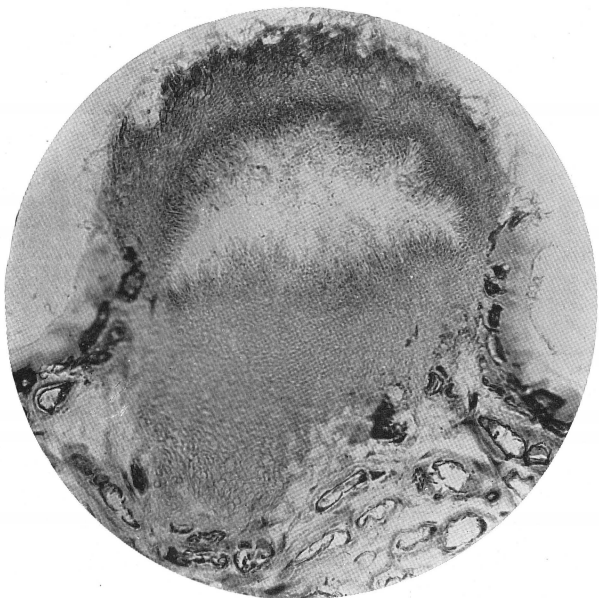


Fig. 40. Pycnidium of *Phoma*. x 180.





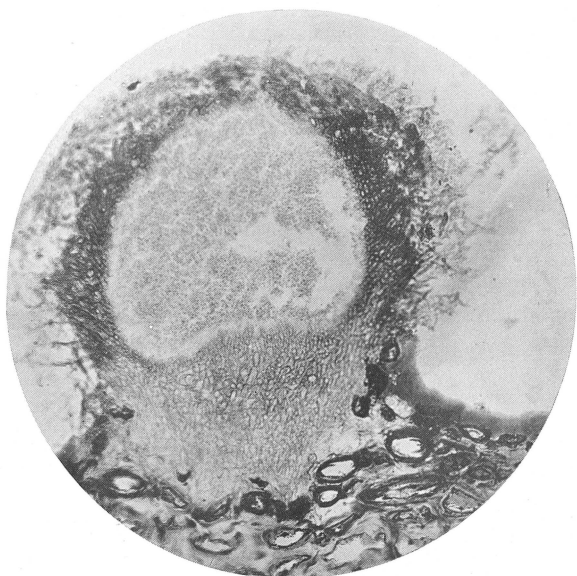


FIG. 41. Mature pycnidium of *Phoma* showing the cavity filled with spores.  $\times 160$ .

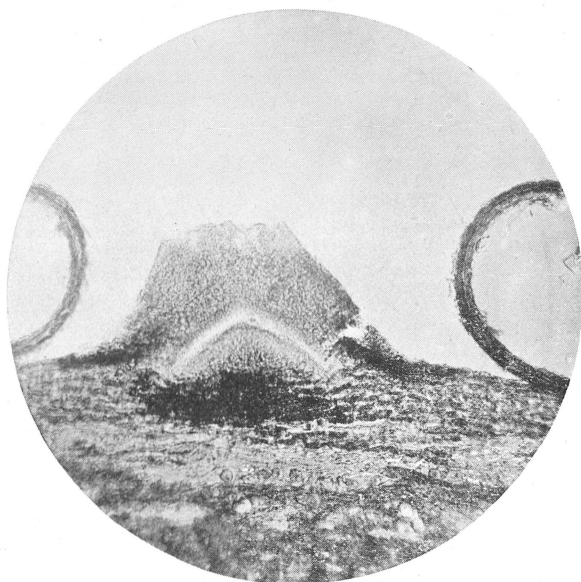


FIG. 42. Pycnidium of *Phoma* from one of the inoculated trees. Note outer layers of bark broken away.  $\times 80$ .



